PN-AAS-616 39686

INTEGRATED APPROACH TO NITROGEN FIXING TREE GERMPLASM DEVELOPMENT

JAKE HALLIDAY[1]

ABSTRACT - The performance of nitrogen fixing trees introduced to new environments depends on proper reconstitution of the symbiotic associations on which the trees rely for their nutrition. Thus selection strategies employed to identify adapted germplasm for particular sites must provide for three-way selection of seed, rhizobia and mycorrhizae. Selected lines must then be multiplied before they can be deployed in varying types of development programs. Special problems are faced in accomplishing these ends with virtually all nitrogen fixing trees. Results and experiences are described which emphasize the importance of parallel selection of plant germplasm and *Rhizobium* strains. In the case of VA mycorrhizae, effective symbioses can occur without specific inoculation. Methods for selecting and multiplying trees and their microsymbionts on a large scale are described and discussed.

Index terms: mycorrhiza technology, *Rhizobium* technology, nitrogen fixing trees, plant selection, seed technology.

AÇÃO INTEGRADA PARA DESENVOLVER GERMOPLASMAS FIXADORES DE NITROGÊNIO

RESUMO - O comportamento de árvores fixadoras de nitrogênio depois de serem introduzidas em novos ambientes depende da reconstituição plena das associações simbióticas, as quais contribuem para a nutrição das plantas. No entanto, as estratégias de seleção empregadas para identificar germoplasmas adaptados para certos locais precisam levar em conta a seleção conjunta das sementes, do rizóbio e das micorrizas. As linhas selecionadas têm de multiplicar-se em grande escala antes de serem utilizadas em vários tipos de programas. Encontram-se graves problemas para alcançar estes fins em quase todos os casos de árvores fixadoras de nitrogênio. Descrevem-se experiências e resultados que enfatizam a importância da seleção paralela de germoplasma de plantas e cepas de *Rhizobium*. Nos casos de micorrizas AV, efetivas simbioses podem ocorrer sem inoculação específica. Apresentam-se metodologias para a seleção e multiplicação de árvores e seus microssimbiontes em grande escala.

Termos para indexação: tecnologia de micorriza, tecnologia de *Rhizobium*, árvores fixadoras de nitrogênio, seleção de plantas, tecnologia de sementes.

INTRODUCTION

Nitrogen fixing trees (NFTs) are being acclaimed for their potential role in agricultural development. This acclaim has stimulated research on fast-growing, nitrogen fixing trees. Before any species

[1] The author is Director of the University of Hawaii NifTAL Project and MIRCEN, P.O. Box O, Paia, Hawaii 96779, U.S.A.

can be put to effective use, whether it be in agriculture, agroforestry, or reforestation, reliable propagation technology is essential. This is a sobering prospect because of the special problems with propagation of leguminous trees. It behoves the research community to anticipate possible future constraints on emergence of a complete utilization technology and to phase the research to be addressed in a balanced manner. This paper discusses just what constitutes the germplasm unit of an NFT and describes strategies for its propagation.

Nitrogen fixing trees potentially benefit from at least two symbiotic relationships with microorganisms. Association with rhizobia confers nitrogen fixing ability. Infection with vesicular arbuscular (VA) mycorrhizae enhances phosphorus uptake. A vigorous NFT at a particular location is frequently a manifestation of an especially effective match between the tree genotype, its symbiotic partner (s) and its environment. In fact, such a tree could be heavily dependent on its microsymbioses for its nutrition. Germplasm explorers need to be aware that later performance of a collected accession introduced to a new location may be below expectation unless a specific effort is made to reconstitute equally effective associations.

Introduced NFT species can fail to encounter fully effective microsymbionts spontaneously in the native microflora, and/or nodulate effectively with available inoculants. Even when the trees are inoculated this may be unsuccessful if inoculant strains do not match the tree's specific requirements. Thus, an integrated approach to germplasm exploration, selection and introduction is warranted. Seeds, rhizobia and mycorrhizae can be viewed as inseparable components of the NFT germplasm unit.

NFT SEED TECHNOLOGY

Relatively few NFT species are self-pollinated. Genetic heterogeneity of most NFT species is problematic at virtually every stage in conventional crop improvement strategies. Heterogeneity complicates germplasm exploration, selection and multiplication, and is confounded by the often lengthy generation times of even the fast-growing NFT species. So formidable is the task that releasing mixtures of seed of tree accessions that are phenotypically similar but which are genetically diverse may be the only practical approach to putting these species to work for development in the tropics.

For self-pollinated NFTs, seed production is relatively straight forward. But in fact, very little research has been done on the specific culture of any NFT for seed production. Most seed "production" is actually the result of collection from natural NFT populations or from plantations established for purposes other than seed production. As the more promising NFTs move closer to being utilized on an extensive scale, seed production technology becomes increasingly important.

Research on management of *Leucaena leucocephala* for optimum seed production has been summarized in a technical manual (Halliday & Billings 1983). The manual covers: sources of foundation seed, orchard design, site preparation, inoculation and planting; weed control, pest and disease control, orchard maintenance, harvesting, and seed cleaning and storage.

In the case of leucaena, seed production per tree can be enhanced by specific management practices such as spacing and regular annual pollarding. Typical seed yields of K 8 giant leucaena under Hawaii conditions are 0.48 kg/tree at 1 m x 1 m spacing, and 1.25 kg/tree at 2 m x 2 m spacing in the establishment year. Older trees in our main orchard are yielding about this same level.

It is difficult to understand why there continues to be a seed shortage of such a prolific seeder as the giant leucaena. A single, three year-old tree at Pukalani, Maui, yielded 16 kg of seed. This amount would be sufficient to plant 500 hectares.

It should be remembered that although a particular NFT might have been selected for its adaptation to a particularly stressful environment, seed production of the species need not be conducted under such conditions. The economics of seed production usually permit seed production to be pursued under conditions that favor seed production. These conditions might be very different indeed from those in which utilization of the tree is proposed.

Seed of cross-pollinated species need to be produced in plantings that are screened off or raised in isolation. The latter is the more practical with NFTs.

In Hawaii, cross-pollination of superior introductions of leucaena species with almost ubiquitous inferior naturalized leucaena is problematic. A novel approach to achieving seed production despite this difficulty has been to exploit leucaena's intolerance of acid soils. Naturalized leucaena is absent in certain acid soil enclaves on the Island of Maui. Thus,pure seed of leucaena is being produced in such an area (Kuiaha site, Humoxic Tropohumult, pH 4.5) after liming only the immediate seed orchard to permite growth of the desired leucaena line.

Seed technology is at its soundest when the foundation seed is homogeneous. This is a real problem with many of the NFTs. Vegetative propagation and tissue culture approaches are perhaps overrated as solutions to this problem.

While it is relatively easy to achieve rooting of stem cuttings of some NFT species, notably those that are used as living fenceposts, survival of the vegetative propagules of most species is highly variable under realistic reforestation circumstances. Also, propagation by stem cuttings has a high water requirement and is labor intensive.

Experiences with clonal propagation through tissue culture of legume species have been largely disappointing. This is especially true of the leguminous trees. There has been only limited research on propagation of NFTs by tissue culture methods. In their work with Hawaii's native *Acacia koa*, Skolmen & Mapes (1976) found that only juvenile tissue (tips of root suckers) gave calluses. These were then stimulated to differentiate shoots and roots and were able to grow independently. *Acacia koa* is one of the very few tree species to have been propagated by tissue culture.

RHIZOBIUM TECHNOLOGY

Genetic diversity of planting material is just one of the features of NFTs that make it necessary to rethink some of the conventional approaches to selection of *Rhizobium* strains for use in inoculants. This section of this paper deals with rhizobial strain selection and inoculant production procedures.

Previous publications by Halliday 1979, 1981 have defined stepwise screening procedures for selecting strains of *Rhizobium* to use in legume inoculants. Most selection procedures for crop legumes stress the matching of specific rhizobial strains with the host genotype. Such a procedure is valid for certain NFTs, but is inappropriate, at least in the short-term, for most NFTs that may be less defined

genetically. Hopefully, in the long-term the host germplasm would be more homogeneous and inoculants could be developed on the basis of matched specific strains.

A second complication for inoculation of NFTs is that vegetative propagation may be necessary for some species. Conventional inoculant methods involve application of rhizobia directly to the propagule or indirectly to the soil. Modifications have not been validated for use with vegetative propagation. Such validation is necessary because delay time between planting and root emergence is much longer with vegetative propagules than in the case of seed germination. The period during which rhizobia are vulnerable to adverse factors is prolonged. There may not be survival of adequate numbers to effectively nodulate the root when it finally emerges.

The following account outlines an accepted approach to the selection of *Rhizobium* strains for use in legume seed inoculants. The procedures described were used successfully in a specific program concerned with the selection of appropriate rhizobia for forage legume introductions in acid, infertile soils of tropical Latin America (Halliday 1979). The principles underlying the approach apply equally well to strain selection for NFTs and some examples of alternative methodologies are mentioned in the text. Individual investigators can modify the techniques and improvise with equipment to suit their own purposes and the facilities available to them provided they take account of the underlying principles of *Rhizobium* strain selection stressed here.

Strain selection is performed to ensure that a legume seed inoculant contains a strain, or strains, of *Rhizobium* capable of forming fully effective, nitrogen-fixing nodules on the legume species for which it is recommended and under the conditions of soil and climate in which the legume crop is grown.

Some characteristics of strains of *Rhizobium* to be used as legume inoculants can be regarded as "essential" whereas others are "desirable", depending on the specific selection objective.

One essential characteristic is the ability to nodulate the NFT of interest in the field conditions under which it is grown. Such strains are referred to as infective. Strains of *Rhizobium* which are infective in the field will usually have exhibited competitive ability if they displaced nodulation by native strains present at the site. They will also have been stress tolerant if they successfully nodulated legumes in soils with excesses or deficiencies in their physical/chemical composition.

A second essential characteristic is that the strain be able to fix sufficient nitrogen to sustain a level of legume production close to, or surpassing, the production possible if the legume were supplied with nitrogenous fertilizers. Such strains are referred to as effective. Strains which are fully effective are usually carbon efficient and hydrogen efficient as well. The "efficiency" of a *Rhizobium* strain is seldom measured during strain selection and use of the term in this context should be avoided. Effectiveness is usually what is meant.

A third essential character of an ideotypic *Rhizobium* strain is that it should perform satisfactorily when subjected to the component processes of commercial-scale inoculant production systems. Inoculant strains must multiply well in bulk culture and be able to mature to high populations in the carrier material.

A fourth essential character is ability to survive well during distribution to, and use by, farmers. Strains should be tolerant to the anticipated maximum temperature that they will encounter. They

must also survive well during the seed/soil inoculation procedures used by farmers. Additionally, they must survive on seed in soil from the time of their application until the emerging legume radicle is susceptible to infection (usually at least seven days). Strains for NFTs will need to survive for even longer periods if they are used with vegetative propagules, and/or to cope with delayed germination. Characteristics which are in the "desirable" category are long-term persistence and fungicide/insecticide tolerance.

Long-term persistence is expected of strains of *Rhizobium* used to inoculate perennial species. Implicit in the concept of persistence is saprophytic competence, a summary term for all those traits that permit a *Rhizobium* strain to live as a stable member of the soil microflora, even in the absence of its legume host. Persistence of strains for annual crop legumes from season to season may be considered a desirable trait in some circumstances, as it obviates the need for inoculation in subsequent years. But there may be cropping systems in which carry-over strains from a previous crop may nodulate a following crop relatively ineffectively and even out-compete effective introduced strains. This can occur in rotations of soybean with peanut and cowpea that nodulate with the cowpea miscellany.

Fungicide or insecticide resistance may be desirable traits when normal practice is to sow legume seeds pre-treated with these substances, some of which are toxic to most stains of *Rhizobium*.

Rhizobium strains do vary widely in the characteristics listed above. Some strains nodulate some genera, or species, or varieties of legumes and not others. This has given rise to the durable, but highly criticized, taxonomy of rhizobia based on their cross-inoculation affinities. Among the strains capable of infecting and nodulating a particular legume, there is great variation in the amount of nitrogen they fix, i.e., variation in effectiveness. There is considerable strain variation in the other listed traits as well and thus an opportunity exists to select superior strains. Unlike higher plants which can be improved through breeding and hybridization, *Rhizobium* improvement is currently practical only by selection from natural populations.

As will be appreciated from the following procedures, the selection of superior *Rhizobium* strains is a lengthy undertaking. Several years of study may be necessary to complete characterization and testing. Given that strains of *Rhizobium* for many legumes, including some NFTs, have already been developed at research labs around the world, it makes sense to obtain and use these, rather than initiate an extensive selection program (NFTA 1983). Selection of rhizobia is only really justified when the specific selection objective cannot be satisfied by strains held in existing collections. Examples of circumstances under which strain selection may be required are as follows:

1. When the legume of interest is an uncommon species for which there is no recommended inoculant strain. This is the "state-of-the-art" for the majority of NFTs.

2. When inoculation of the particular legume with recommended strains of *Rhizobium* under field conditions fails to give adequate nodulation and nitrogen fixations. This can occur if the legume variety is different from that with which the inoculant strain was developed, or if the soil and climatic conditions vary from those under which the inoculant was developed.

A step-wise selection procedure will be described for the development of a *Rhizobium* strain recommendation for legumes planted under a particular soil condition. This approach is unconventional in the sense that strains of *Rhizobium* in current use as legume seed inoculants are developed for the

species of legume with which they will be used, rather than the soil type in which the legume will be grown.

In the technologically advanced countries, it is normal farm practice to modify soil conditions to be suitable for a particular crop. It is not unreasonable, therefore, to expect a rhizobial inoculant for a legume species to perform well wherever that legume is grown. In the developing nations, however, soil amendment is minimal or not practiced at all, and crop plants are often grown under stresses of adverse soil factors that cannot be economically alleviated. For most utilizations, e.g., reforestation, NFTs will be introduced to unamended soils. It may be unreasonable to expect that a single strain of *Rhizobium* will perform equally well as an inoculant in the wide array of soil types under which its host legume is grown in the tropics. One reason that legume inoculation is not widely successful in developing countries is that available inoculants obtained from the U.S., Australia, or elsewhere do not have strains selected for, and adapted to, the extremes of soil stress encountered in the tropics (Halliday 1981b).

There is a widely held view that strain selection and legume inoculation have little potential for improving yields of tropical legumes since tropical legumes are not specific in their *Rhizobium* strain requirements, and because suitable rhizobia occur universally in tropical soils. Spontaneous nodulation of NFT species in their natural environment creates an impression that specific inoculation is not called for.

There are a few notable exceptions, such as soybeans and leucaena, and thus two categories of tropical legumes were recognized. The promiscuous (P) group can be nodulated by a wide array of strains of tropical rhizobia. The specific (S) group requires specific rhizobial strains for nodulation. The majority of tropical legumes were judged to belong to the P group and it has been generalized that it is unnecessary to inoculate these legumes with rhizobia, as no benefit would be expected.

The grouping of tropical legumes simply as S or P types is no longer tenable nor useful. Many tropical legumes previously placed in the P group are now known to form fully effective (i.e., high nitrogen-fixing) symbioses with only a few strains out of the diverse array of rhizobia that can nodulate them. Thus, a distinction is drawn between this promiscuous-ineffective (PI) group of legumes and the promiscuous-effective (PE) group (Date & Halliday 1980). Studies of the *Rhizobium* affinities of the tropical forage legumes, for example, reveal that a majority of them are in the PI group, suggesting a potential for increasing their production by providing appropriate strains of rhizobia.

The important role played by stress factors of tropical soils as modifiers of symbiotic performance is now well recognized (Halliday 1981b). Thus, tropical legumes can and do benefit from inoculation when strains are selected specifically for the particular variety of legume being planted and for tolerance of the soil conditions in which that legume is to be grown.

No strain selection program should be undertaken without clear definition of the specific selection objective(s). The methods of selection employed may need to be modified to suit the objective. The specific selection objective for which the procedures that follow were developed was to select strains of *Rhizobium* able to nodulate and fix nitrogen in association with acid tolerant legume accessions being introduced to the acid, infertile soils of Latin America.

Successful selection of superior rhizobia is favored if the number of strains from which the selection is made is large and diverse. The most meaningful test of *Rhizobium* performance is field evaluation

since this is an integrated appraisal of the various traits that make up a successful inoculant strain. However, the management of field trials to select rhizobia is difficult and costly, even when the number of strains under test is small. Multi-stage screening procedures that progressively eliminate undesirable strains from as initially high number of contenders yielding a relatively small number of promising strains for testing at the field level. This is one way to reconcile the requirements that selection be from a diverse genetic base, and that strains also be assessed under field conditions.

It is advisable to include in the screening procedure strain of *Rhizobium* that originated from a diverse array of host plant germplasm and that are representative of diverse geographic regions. But some reduction of the number of strains can be made based on what is known from other selection programs. In general, rhizobia isolated originally from the same genus, and sometimes species, as the legume for which a superior strain is being sought emerge from selection programs as the best strains for use in legume inoculants. Also, when the specific selection objective includes tolerance to a particular soil stress or climatic condition, rhizobia isolated from legumes growing under those conditions are the most likely to be rated highly in the selection process. Hopefully, there is a *Rhizobium* collection or collections of authenticated strains of known origin available to the investigator. Otherwise, a suite of strains has to be assembled. Present status of recent strain acquisition for NFTs at NifTAl is reflected in Appendix I. Only after checking whether likely strains are available from existing *Rhizobium* collections, such as the *Rhizobium* Germplasm Resource at NifTAL, should collection and isolation of new strains be contemplated. Detailed procedures for the collection, isolation, purification, authentication, characterization, and preservation of strains of *Rhizobium* are described elsewhere (Halliday 1979, 1981). Pre-selection of strains with suitable background should aim to generate a cluster of 50-100 rhizobia that will feed into Stage I of the strain selection procedure.

Stage I - Screening for Genetic Compatibility: In this stage, strains of *Rhizobium* are screened for ability to nodulate the legume of interest. The test used involves a high degree of bacteriological control and is suited to handling large numbers of strains. The system most commonly used is based on growth tubes in which seedlings are raised in a solid, nutrient medium under artificial illumination. Seeds must be surface sterilized, usually with concentrated sulphuric acid, hypochlorite, or acidified mercuric chloride. They are pre-germinated in inverted, sterile perti dishes of water agar. When the radicles are 3-5 mm long, uniform seedlings are transferred aseptically to tubes containing agar deeps (or slants). Tubes are routinely 2.5 x 25 cm, capped with a plug of muslin-wrapped cotton wool. Aliquots of 1 ml of suspension of the test strains are added to each tube either at transplanting or 3-5 days later. At least three replications of each strain treatment are essential and five are preferred. Roots of seedlings should be shielded from light. Alternatively, tubes may be wrapped in aluminum foil. Two control treatments are required. In one case the plants are "inoculated" with sterile water only (uninoculated control) and in the other case they are provided with 70 ppm nitrogen as ammonium nitrate (or potassium nitrate) solution (plus nitrogen control). Tubes are scored at intervals for the presence or absence of nodules. With many tropical legumes, tumor- or callus-like outgrowths can occur on roots of seedlings raised in growth tubes. These outgrowths occur in the presence or absence of rhizobia and are not nodules. They cannot be distinguished from nodules by eye. Plants should be harvested from tubes and checked under a binocular microscope for real nodules. "Apparent" nodules lack structural organization and leghemoglobin. Timing of the harvest varies depending on legume species but will usually be about 35 days after sowing.

Some investigators place significance on other data taken on plants grown in growth tubes. "Earliness to nodulation" may be of some value. It is inappropriate, however, to attribute relative nitrogen fixation effectiveness to strains based on nitrogen accumulation in plants raised under such

artificial conditions. The root medium and atmospheric composition within plugged test-tubes differ from those which the plants require for optimum performance and may constrain expression of nitrogen-fixing potential.

Alternate methodologies are required for large-seeded species that quickly become cramped in growth tubes. These include the use of growth pouches or "Gibson" tubes. Growth pouches are made of autoclavable plastic and have an absorbent towel insert. Seedlings germinate in a fold (or are pre-germinated and transplanted into the fold) at the upper rim of the pouch. Roots develop within the pouch nourished by a nutrient medium, and plant tops grow in the open air. The method offers the advantage that effective nodulation can be reliable determined, but caution in attributing relative effectiveness of strains on a pouch test basis is necessary. Modifications of the method include subdividing the pouches with heat bonding to permit a single pouch to be used for several strain treatments, or replications of the same treatment.

In the case of "Gibson" tubes, the tube contains a long agar slant that reaches to the upper rim of the tube, and are filled to the rim with liquid medium or sterile water. They are capped with aluminum foil. Radicles of pre-germinated seedlings are entered through a small orifice in the aluminum. The roots develop inside the tube and the plant tops grow outside the tube. The method offers similar advantages to those of pouches, namely that effective nodulation shows up readily. Modifications of "Gibson" tubes include omission of the liquid phase or half filling the tubes.

Obviously, nodulation in the uninoculated control treatments in Stage I raises concern about inadequate bacteriological control and invalidates the experiment.

Some texts advocate dedication of entire light rooms for the culture of plants in growth tubes. Most workers will find a low cost system of racks and portable fluorescent tubes more than adequate for their needs. Such a system is highly flexible and can be readily modified to serve for pouches or "Gibson" tubes that require overhead illumination. The issue of light quality has been overplayed. Regular domestic fluorescent lamps have served satisfactorily in the screening procedure described here.

Stage II - Screening for Nitrogen Fixation Effectiveness: In this stage the objective is to rank infective strains from Stage I in order of potential nitrogen fixation effectiveness with the legume species/cultivar of interest. Theoretically, in this test there should not be any factors limiting growth of the legume except nitrogen, so that full expression of each strain's nitrogen fixation effectiveness is possible. In practice, it is assumed that the nutrient regime and other aspects of growth conditions are not limiting, even though there are known examples of legumes for which standard conditions are not non-limiting. Sand jar assemblies are used in this test because they permit more realistic growth conditions than tubes, pouches, etc., but retain the high degree of the bacteriological control which is still essential if valid results are to be expected.

The Leonard jar is one example of such a sand jar assembly. Watering is the most common source of contamination in *Rhizobium* strain testing in pots and in the field. Leonard-type sand jars greatly reduce the frequency of watering and are, therefore, less prone to contamination. Sand jars are easily constructed from locally available materials, but have the disadvantage that sterilizing them requires a very large autoclave.

As with growth tubes, surface sterilized pre-germinated seeds are sown in the sand jars. Four seedlings are allowed to establish and thinned later to two by snipping off the tops. Drops (standardized

rate) of suspensions of strains of *Rhizobium* are added to seedlings in the jars five days after sowing (one strain per jar). Plants are harvested destructively at a time after sowing that depends on the legume species under test. Usually 60 days after sowing is appropriate.

Data taken on sand jar experiments vary from investigation to investigation and include the following:

- nodule number
- nodule dry weight and/or fresh weight
- nodule color
- nodule distribution
- total plant fresh/dry weight
- top weight (fresh/dry)
- root weight (fresh/dry)
- acetylene reduction rate
- percentage N in tissues
- total N produced

Of these, total N produced is the most meaningful integration of nitrogen fixation effectiveness over time and as this is highly correlated with total plant dry weight, a reliable measure of relative effectiveness of strains of *Rhizobium* is possible with nothing more sophisticated nor costly than a common balance.

The main problem encountered with this test relates to overheating in greenhouses or growth rooms where the experiments are performed. Most of the sand jar trials observed by this author in the tropics are, in fact, selecting high temperature tolerant rhizobia at the same time! Other problems relate to the occasional failure of the irrigation from beneath which depends on capillary rise, and breakage of glass components in autoclaving and handling.

Strains are ranked on the basis of their yields in Stage II. The demarcation of effectiveness categories is somewhat subjective, but nevertheless useful. Strains are assessed relative to the uninoculated control and the nitrogen control and described as (in ascending order of merit) parasitic, ineffective, partially effective, moderately effective, or fully effective.

Ordinarily, about 30-50 strains would be evaluated at Stage II in Leonard jars. Three replications are essential and five are preferred. The top ten strains are chosen for further screening at Stage III.

The principal merit of Leonard jar trials is that data on the potential effectiveness of strains of *Rhizobium* with a particular legume tend to be upheld in independent screening trials by other investigators. Thus, researchers can exchange information that is stable and demonstrable on the nitrogen-fixing potential of strains. Pot and field trials, on the other hand, give information of the plant/*Rhizobium* soil interaction that may or may not be repeatable at other locations.

Stage III - Screening for Symbiotic Effectiveness Under Physical, Chemical and Biological Stresses of Site Soils: The fully effective nitrogen fixation effectiveness expressed under Stage II conditions will not necessarily be upheld under real field conditions. Thus, before selecting a final cluster of three strains of *Rhizobium* for field evaluation, it is advisable to subject a larger group (ten) of potentially effective strains to some of the physical chemical and biological stresses of soils for the inoculant is being developed. This stage is particularly useful if the specific selection objective (s) includes adaptation to a particu-

lar stress, such as soil acidity. Stage III also has a value in selection programs for "non-stress" soils. In Stage II sand jar evaluation, test strains did not have to compete against native rhizobia.

This third stage involves a pot experiment in which strains are tested with the host plant and production related to that of uninoculated control plants and nitrogen fertilized plants. Soil is collected from the plough layer and mixed to uniformity to produce a homogeneous experimental material. Unsterilized soil is used. Soil may be amended at fertilizer rates equivalent to field practice, but only the nitrogen control plants receive nitrogen (equivalent to 100 kg N/ha). Procedures for calculating the fertilizer additions are detailed elsewhere. Not all soils behave satisfactorily in pot experiments and other amendments may be necessary, particularly with heavier soils. The following should be considered:

1. Sieving to remove large soil aggregates and stones.

2. Addition of high carbon ratio residues such as bagasse at 1-2% (dry weight basis) to counter balance excessive mineralization of nitrogen resulting from soil handling.

3. Addition of volcanic cinder, vermiculite, or other materials to improve soil aeration and drainage.

Sowing procedure and inoculation is the same as for sand jars in Stage II. About 6-8 seedlings are planted and thinned to 2-4 plants/pot, depending on the species. Thinning is by snipping off the plant tops, rather than pulling entire plants from the soil. Size is optional, but 20-25 cm in diameter is usual. Six replications of each treatment are required.

Precautions against cross-contamination in this stage are essential. Watering, which in greenhouses in the tropics is needed daily, is the primary source of contamination. It can be minimized by:

1. Filling pots so that the soil level is 3 cm below the pot rim.

2. Watering gently to avoid splashing.

3. Using grid or mesh benches instead of solid benches, so that pots can drip through onto the floor.

4. Raising pots on supports (such as petri dish lids) so that there can be no water flow on the bench surface from the emergent roots from one pot to those of another.

5. Assigning watering to a single, informed individual.

Other precautions include avoidance of overheating of the roots and nodules in pots and minimizing non-treatment effects. Pots should be set up in a randomized, complete block design but not re-randomized thereafter because of the overriding problem of contamination through handling.

As with sand jars, plant dry matter production is the most meaningful parameter to be determined and is the basis for ranking strains. The top three strains are promoted to Stage IV.

Stage IV - Single Location Evaluation of Strains of *Rhizobium* and Inoculation Methodology Under Field Conditions: Strains emerging from Stage III are evaluated for nodulation and nitrogen fixa-

tion under field conditions. Although the preferred measure of the response by a legume to inoculation with the test strains of *Rhizobium* is grain yield (dry matter production in the case of forages), there are many factors which, under field conditions, can prevent differences in nitrogen fixed by the strains being translated into differences in yield. Therefore, field trials should include a mid-season harvest to determine dry matter production. Plot size should be sufficient to house two fully bordered harvest areas. The standard plot layout used in the International Network of Legume Inoculation Trials (Halliday 1981b, Date & Halliday 1980) is recommended.

When the specific selection objective includes overcoming soil stress, the field trial at Stage IV can amalgamate the strain selection approach and other strategies for overcoming the stress. In this case, several inoculation methods were appraised for their ability to overcome the effect of acid soil stress on nodulation. Simple seed inoculation with an aqueous suspension of peat-based inoculant containing the test strains was one treatment. Others involved pelletting the inoculated seeds with finely-milled lime or rock phosphate. These treatments were compared to control plots of uninoculated plots and plots fertilized with nitrogen. The comparison between these treatments is most valid, in a scientific sense, when there are no other factors limiting plant growth. But the comparison is most realistic when the level of agronomic inputs is economically feasible and similar to that used by farmers in the region where the legume is grown. In the procedure adopted, the scientific validity was considered of lesser importance than the need to be realistic and a minimal blanket fertilization of elements other than nitrogen was applied. Three replications of the treatments were established in a randomized complete block design. Experience has indicated that four replications are desirable.

Precautions against cross contamination are of paramount importance. Common pathways of contamination are:

1. Careless handling of inoculated seed at planting time.

2. Use of field implements without sterilizing them between plots.

3. Tramping from plot to plot (by laborers, animals, visitors, etc.).

4. Run-off and other drainage problems caused by poor site selection.

The best *Rhizobium*/inoculation method combination is then selected and subjected to further testing in Stage V. It could be justified to produce and use legume inoculant based on Stage IV evidence, but there remains the risk that the selected strain will be a successful inoculant only in the specific soil and climatic conditions under which it is selected. A further stage is essential to determine the range of suitability of inoculant developed for a single location in Stage IV.

Stage V - Multi-location Testing of the Response to Inoculation with Selected *Rhizobium* Strains: A standard design developed for the International Network of Legume Inoculation Trials (INLIT) is available for those contemplating multi-location trials on the response of legumes to inoculation with selected strains of *Rhizobium* (NifTAL 1982). One of the major constraints to fuller utilization of legume inoculation in the tropics is that there has not been convincing demonstration on a wide scale that yield increases will result with local legume varieties under local soil and climatic conditions (Date & Halliday 1980). Stage V trials can assist in deriving the data necessary for predicting more reliably whether a legume will respond to inoculation or not.

The trial has three basic treatments: plants inoculated with *Rhizobium;* plants not inoculated; and plants nc inoculated but fertilized with nitrogen. The comparison is made at two fertility levels which, for con nience, shall be referred to as "farm fertility" and "maximal fertility". Fertilizer levels are determined on the basis of information available locally.

With three treatments at two fertility levels replicated four times, a 24 plot, randomized, complete block design results. The treatments in the first replication can be deliberately arranged to serve as a demonstration in which the treatments that are most frequently compared are located side-by-side to facilitate visual observation of treatment differences. Plot arrangement is the same as for Stage IV. Experimental layout is as in the International Network of Legume Inoculation Trials (NifTAL 1982). Row spacing, planting distance, and seed depend on the legume in question and plot size will necessarily be bigger for NFT inoculation trials.

The plus nitrogen control plots receive 100 kg N/ha but in two doses. At planting, 25 kg N/ha are applied and 75 kg N/ha added 4-5 weeks later in the case of grain legumes. With forages the 25 kg/ha are applied at planting and 25 kg N/ha applied after each cut (approximately three month intervals).

It is best to sow the "uninoculated" and "nitrogen fertilized" plot first. Only after the seeds in these plots have been covered are the inoculated seeds prepared for sowing in the remaining plots. This minimizes the risk of contamination of the plots that are not to receive rhizobia.

Stage V trials can be used to characterize selected strains for competition and persistence if the inoculant strain is "marked" serologically or with antibiotic resistance. Such strains of *Rhizobium* can be detected in the nodule population and their ability to compete against strains native to the site determined. These strains can also be detected, if present, in the soil in following seasons, or in the nodule populations of subsequent legume crops sown uninoculated.

The International Network of Legume Inoculation Trials (INLIT), coordinated by the University of Hawaii NifTAL Project, is available for 17 agriculturally important tropical legumes including the NFT *Leucuena leucocephala.* Inoculants developed for INLIT each contain three serogically distinct, effective strains of *Rhizobium* from diverse geographic and host germplasm backgrounds (NifTAL 1982). Each INLIT is potentially an ecological study of the relative performance of the three exotic strains between themselves and in competition with indigenous soil strains. It is also a long-term persistence trial. A mixed inoculant of six marked strains is now offered for NFT research.

For some specific selection objectives, the development of rapid screening procedures may reduce the time taken to develop a reliable inoculant strain, or may greatly increase the likelihood of successful inoculant strains emerging from the step-wise screening previously described. For example, in the case of selection of rhizobia for acid, infertile soils, a laboratory prescreening that preceded the Stage I test greatly increased the range and numbers of strains that could be addressed. It eliminated effective strains predestined to fail in the field but which would have passed through Stages I, II and possibly III consuming time and resources. The prescreening test was based on the reasonable assumption that for a strain of *Rhizobium* to be a successful inoculant for legumes grown in acid soils, ability to multiply well at low pH is an essential trait. Synthetic media were developed that tested ability to multiply at low pH, and only those strains passing the test were fed into the step-wise screening program (Date & Halliday 1979). Investigators may find it useful to adopt rapid prescreening steps for their own objective(s).

As with any screening program, there is always the risk that discarded materials that could not be accommodated in the later stages would have performed well in the field. In the procedure described,

the stage-to-stage transition that is most problematic is that from Stage II to Stage III. Rankings of strains in sand jars do not necessarily hold up when subjected to the stresses of site soils. Although ten fully effective strains are passed across from II to III, examples have occurred in which as few as three of the strains could nodulate at Stage III and only one of these was effective.

When dealing with uncommon legume species, such as NFTs, an investigator should be concerned about whether the routine media used in Stage I and Stage II are, in fact, non-limiting on growth of the legume plant so that *Rhizobium* characters can be expressed. As an example of this, it was found that *Stylosanthes capitata*, a legume with high tolerance to soil acidity factors and native only to acid soil regions of South America, could not be nodulated by any one of more than 100 *Stylosanthes* isolates (including many specifically from *S. capitata*) tested at Stage I. Nor would *S. capitata* grow in Stage II. Only when the growth medium was acidified to a pH lower than 5.0 and the Ca and P levels lowered ten-fold would the plant nodulate and grow.

Even though the screening procedure is lengthy, attempts to short-cut the sequence are ill-advised. Recommendation of strains of *Rhizobium* for NFT inoculation without first performing field trials similar to those described in Stage IV and Stage V is risky in the face of accumulating data that indicate that site variation in performance of selected strains is common (Halliday 1983).

The underlying objective of inoculation technology is to place such high numbers of preselected strains of rhizobia in the vicinity of the emerging root that they have a competitive advantage over any indigenous soil strains with lesser N-fixing ability in the formation of root-nodules.

Inoculation technology involves: selection of strains of rhizobia that are compatible and effective N-fixers with particular legumes; multiplying selected strains to high population densities in bulk cultures; incorporating the liquid rhizobial cultures into a carrier material (usually finely milled peat) for packaging and distribution; and finally, coating the seeds of legumes with the carrier or implanting the soil with the inoculant directly into the seed drill.

In addition to the selection criteria already described, inoculant strains need an ability to grow and survive in peat inoculants.

The host genotype interacts with the infecting strain of *Rhizobium* in determining the level of nitrogen fixation with the host playing the dominant role. Thus, two sources of variation (plant and *Rhizobium* strain) can be exploited in selection programs. Most commonly, though, the plant is selected independently and a suitable strain sought thereafter, thus allowing only for exploitation of strain variability. The range of specificities of host genotype interactions is well illustrated by soybean and the African clovers.

Such specifities give three options in the approach to selection of strains for inoculants: numerous inoculants, each with a highly effective strain for individual species; "wide-spectrum" strains that vary from good to excellent in nitrogen fixation with a range of legumes; or multiple-strain inoculants containing the best strain for each host species. There may be a conflict between the option that would be chosen for commercial expediency and that which is scientifically excellent. In Australia "wide-spectrum" strains are used when these are available, but there is increasing use of specialized inoculants with specific strains for individual hosts. Despite findings which suggest that multi-strain inoculant should be avoided because of possible antagonistic and competitive effects in culture and the likelihood of competition in

nodule formation from the less effective strains, this is the approach used successfully by the U.S. inoculant industry.

The number of NFT species to be addressed exceeds 1,000 (Appendix I). An expert group reduced the list of NFTs of highest priority to 44 species. Inoculant for these is needed even before development of specialized inoculants can be completed, and NifTAL advocates a multi-strain inoculant incorporating wide-spectrum, fast- and slow-growing rhizobia. Results to date with this inoculant vindicate this approach (Halliday & Somasegaran 1983).

Most legume inoculants are prepared by adding liquid cultures of *Rhizobium* to a finely-ground carrier material such as peat. Although mixtures of peat with soil or compost mixtures, lignite, coir dust and some other organic materials have been used, peat has proven to be the most acceptable carrier worldwide. Agar, broth and lyophylized cultures are not recommended because of the very poor survival of these forms of the inoculum on seed.

Peat cultures can be prepared in two ways. Either ground (milled) peat is mixed with a high variable count (more than 109 rhizobia/ml) broth culture in sufficient volume to provide the minimum number of *Rhizobium* acceptable for use, or sterilized peat is inoculated with a small volume of culture and incubated to allow multiplication of the rhizobia in the carrier (Somasegaran & Halliday 1982). The choice of method will depend on two main factors: the survival of the rhizobia in peat in numbers high enough to meet a minimum standard of quality; and the availability of suitable, sterilizable containers and sterilizing facilities. The two factors that most affect survival of rhizobia in peat are temperature of storage and sterility of the peat. There are differences among species and also between strains of the same species of *Rhizobium* in their ability to survive well in peat.

Like all biological products, legume inoculants are prone to loss of quality owing to variation in the organism concerned and from unforeseen factors affecting some aspect of growth or survival. Quality control is an indispensable component of inoculant technology. In Australia, large scale manufacture of legume inoculants is by private enterprise, and an independent (government) control laboratory maintains and supplies recommended strains of *Rhizobium* to the industry. This laboratory checks strains annually for ability to fix nitrogen, assesses quality of cultures during and after manufacture, and conducts such research as may be necessary to overcome problems associated with production and survival in the final product. In the U.S., the industry is free to select its own strains and official control ensures only that the product can form nodules on the legume for which it is recommended.

Although control of quality of inoculants is primarily in the manufacturer's interest and therefore his responsibility, power of control by external bodies provides protection from less scrupulous operators and genuine failure of a strain beyond the manufacturer's control. Not all countries back their control labs with legislation. In Australia, this control extends to holding stocks of the strains used in inoculants. This is not the case in the U.S

In addition to assessment of quality throughout manufacture, it is important to monitor quality of product in retail outlets. Standards acceptable at his level may vary from that at manufacture and between countries. It is important that standards be realistic and within the capability of manufacturers, yet ensure that sufficient viable rhizobia are appplied to the seed to provide a satisfactory inoculation. This can be as few as 100 rhizobia per seed but in cases of severe environmental stress as high as 10,000 or even 500,000. Despite several attempts, it has not been possible to gain acceptance of a universal set of standards for inoculant products.

The first attempts at inoculation involved the transfer of soil from one field to the next, but with the isolation of the organisms responsible for nodule formation, artificial cultures soon replaced the laborious soil transfer technique. The usual inoculation technique is to treat seed just before sowing either with a dust or with a slurry in water or adhesive solution. Adhesives such as gum arabic and substituted celluloses not only ensure that all the inoculum adheres to the seed surface but also provides a more favorable environment for survival of the inoculant. Pelleting of seed with finely ground coating materials such as lime, bentonite, rock phosphate and even bauxite have been used to protect rhizobia during their time on the seed coat. Pelleting is a simple on-farm technique but custom-pelleted (by seedsmen at farmer's request) and preinoculated seed is now more popular. This latter procedure is potentially able to provide high populations of rhizobia on the seed for long periods of time (one growing season to the next) but has not yet been fully developed or exploited. Most preinoculation procedures are based on multiple coatings, alternately of adhesive and finely ground pelleting materials as used in simple pelleting. The peat inoculant is included as one (or more) of these coating layers.

Soaking seeds in a broth suspension and then exposing them to either high pressure or vacuum to impregnate the rhizobia into or below the seed coat has not proven successful. Theoretically, rhizobia introduced in this way would be protected from drying and other adverse environmental conditions, but the quality of products produced commercially has been variable to very poor. It is, in fact, an indictment of the research workers in such inoculant methods that 25 years have yielded so little progress in an area that has so much to offer for those concerned with the practical aspects of agricultural microbiology. The preinoculation technique is particularly applicable in a development setting because a high quality and reliable product could be marketed by a manufacturer or seed distributor without the need for farmer involvement in legume inoculation.

An alternative to pelleting and preinoculation in recent years has been the use of concentrated liquid or solid granular peat culture. These are sprayed or drilled directly into the soil with the seed during planting. Suspensions of rhizobia either as reconstituted frozen concentrates or suspensions of peat inoculant can be applied with conventional equipment. Similarly, granulated peat inoculants can be drilled in from separate hoppers on the drilling equipment. These methods have been especially successful for introducing inoculant strains into situations where there are large populations of competing naturally occurring soil rhizobia or in cases of adverse conditions such as hot-dry soils and where insecticide or fungicide seed treatment precludes direct seed inoculation. Solid inoculant, also known as granular or "soil implant" inoculant, is advantageous also where seeding rates for crop legumes of 70-100 kg/ha make on-the-farm inoculation logistically impractical. It is these granular inoculants that would appear most appropriate also for use with NFTs.

VESICULAR ARBUSCULAR MYCORRHIZAL TECHNOLOGY

Micorrhizal infection of many NFTs occurs spontaneously in field soils in Hawaii. Thirty species of NFT from the NFT Germplasm Resource held at NifTAL were sown in Hamakuapoko soil (Typic Haplustoll, pH 6.9) on Maui. Naturalized vegetation at the site includes spiny amaranth (*Amaranthus spinosa*), some wild Cruciferae, and the legumes *Indigofera fructicosa* and *Leucaena leucocephala*. All but one of the introduced species were observed to be heavily infected with VA mycorrhizae by 12-16 weeks after planting (Table 1). This suggests that specific inoculation of NFT seeds with VA mycorrhizae may be unnecessary.

It has been shown that leucaena seedlings raised under nursery conditions did not become infected spontaneously in a peat moss/vermiculite rooting medium. The medium had not been sterilized, but it

is presumed that the source materials were largely free of mycorrhizal spores. Following transplanting to Hamakuapoko field soil, seedlings became progressingly infected with VA mycorrhizae and after 8 weeks attained a level of infection (95%) typical of field grown leucaena (Table 2).

TABLE 1. Observations on presence or absence of nodules and the degree of VA mycorrhizal infection on roots of leguminous trees introduced to Hamakuapoko soil.

NFT N°	Species	Nodulation	VA Mycorrhizal infection
101	*Acacia albida*	yes	90%
106	*Acacia holoserica*	yes	42%
171	*Acacia mangium*	yes	80%
152	*Acacia mellifera*	yes	76%
103	*Acacia nilotica*	yes	91%
154	*Acacia nubica*	yes	88%
157	*Acacia seyal* var. *seyal*	yes	99%
338	*Albizzia chinensis*	yes	96%
181	*Albizzia falcataria*	yes	94%
185	*Albizzia julibrissin*	yes	91%
182	*Albizzia moluccanna*	yes	97%
161	*Calliandra calothyrsus*	yes	96%
321	*Cassia siamea*	no	100%
320	*Enterolobium cyclocarpum*	yes	98%
127	*Julbernardia globiflora*	no	28%
569	*Leucaena leucocephala*	yes	95%
114	*Prosopis africana*	yes	90%
116	*Prosopis juliflora*	yes	94%
323	*Samanea saman*	yes	100%
303	*Sesbania grandiflora*	yes	86%
120	*Tamarindus indica*	no	98%

(From Halliday & Nakao 1982).

TABLE 2. VA Mycorrhizal infection of *Leucaena leucocephala* established by direct seeding or by transplanting (Data of P. Nakao, unpublished).

Plant age (days)	VA Mycorrhizal infection (as percentage)			
	Direct seeded		Transplanted	
21	51	in	0	
49	74	field	0	nursery
56	82		0	
63	95		2	
70	> 95	in	43	in
84	> 95	field	61	field
112	> 95		95	

– – nursery plants raised in dibbling tubes in a non-sterile peat moss-vermiculite mixture (3:5 ratio by volume) and transplanted to the field on day 60.

—— Typic Haplustoll, pH 6.9, Hamakuapoko, Maui, Hawaii.

Further research is necessary to determine whether other species are readily infected with native VA mycorrhizae and whether VA mycorrhizae are ubiquitous in tropical soils.

None of the above considerations precludes the possibility that at some point in the future, mycorrhizal inoculant technology might emerge based on displacement of relatively ineffective native strains by selected strains that are more highly effective phosphorus absorbers. But for the present, inoculation of NFTs with mycorrhizae seems unnecessary. This is perhaps just as well because inability to raise VA mycorrhizae in the absence of a host plant remains a serious obstacle to large-scale production of VA mycorrhizal inoculants.

ACKNOWLEDGEMENTS

Technical support: D. Billings, J. Dozier, J. Mann and P. Nakao.

Financial support: USAID Contract N° DAN-0613-C-00-2064-00
USAID Grant N° DAN-5542-G-SS-2094-00
The National Academy of Sciences (through a W.H. Donner Foundation grant)

REFERENCES

DATE, R.A. & HALLIDAY, J. Relationships between *Rhizobium* and tropical forage legumes. Adv. Legume Sci., Kew, England, 1980. p.579-601.

DATE, R.A. & HALLIDAY, J. Selecting *Rhizobium* for acid, infertile soils of the tropics. Nature, 277:62-4, 1979.

HALLIDAY, J. Agrotechnologies based on symbiotic systems that fix nitrogen. Office of Technology Assessment Workshop on Innovative Biological Technologies for Lesser Developed Countries, 1981. p.243-73. (Paper, 5).

HALLIDAY, J. Biological nitrogen fixation: Its potential in tropical soils. EMEJUAIWE, S.O. et al., eds. Global impacts of applied microbiology. 1981b. p.73-84. Proceedings of Sixth International Conference.

HALLIDAY, J. Field responses by tropical pasture legumes to inoculation with *Rhizobium*. Pasture production in acid soils of the tropics. Colombia, CIAT, 1979. p.123-37.

HALLIDAY, J. *Rhizobium* relations of tropical legumes in tropical soils and implication for transfer of agrotechnology. University of Hawaii, 1983. (Benchmark Soils Project, Tech. Rep., 7). Proc. Workshop on a Multidisciplinary Approach to Agrotechnology Transfer.

HALLIDAY, J. & BILLINGS, D. *Leucaena* seed production systems for third world countries. NifTAL Project. Maui, Hawaii, 1983.

HALLIDAY, J. & NAKAO, P.L. The simbiotic affinities of woody species under consideration as nitrogen-fixing trees. NifTAL Project. University of Hawaii, 1982. 85p.

HALLIDAY, J. & SOMASEGARAN, P. Nodulation, nitrogen fixation and *Rhizobium* strain affinities in the genus *Leucaena*. In: PROC. WORKSHOP ON LEUCAENA RESEARCH IN THE ASIAN-PACIFIC REGION. IDRC, 1983. p.27-32.

NFTA. Resource documents on nitrogen fixing trees. Waimanalo, Hawaii, 1983.

NifTAL. International Network of Legume Inoculation Trials. NifTAL Project, Maui, Hawaii, 1982.

SKOLMEN, R.O. & MAPES, M.O. *Acacia koa* Gray plantlets from somatic callus tissue. J. Heredity, 67:114-5, 1976.

SOMASEGARAN, P. & HALLIDAY, J. Dilution of liquid *Rhizobium* cultures to increase production capacity of inoculant plants. Appl. Environ. Microbiol., 44:330-3, 1982.

APPENDIX I

MASTERLIST OF WOODY SPECIES UNDER CONSIDERATION AS NITROGEN-FIXING TREES

A resource document prepared for the Bellagio Workshop on Nitrogen-Fixing Tree Germplasm

Notes:

1. The NFT Masterlist includes:

   -- all woody species of the legume family even though confirmation that they individually nodule and fix nitrogen may be lacking.

   -- all species of all other genera in which a species has been confirmed to nodulate or fix nitrogen.

2. The masterlist is abstracted from a larger data base maintained by the University of Hawaii NifTAL Project. The complete data base includes a general characterization of each species, and specifies its microsymbiotic affinities, both rhizobial and mycorrhizal. The complete data base also cites the scientific literature that substantiates that a listed species does or does not fix nitrogen.

3. The Masterlist is actually the first section of a fuller publication available directly from NifTAL (P.O. Box O, Paia, Hawaii 96779, USA):

HALLIDAY, J. & NAKAO, P.L. **The symbiotic affinities of woody species under consideration as nitrogen-fixing trees**; NifTAL Project. University of Hawaii, 1982. 85p.

Y *Acacia abyssinica*
Y *Acacia acinacea*
Y *Acacia acuminata*
N *Acacia adenocalyx*
Y *Acacia adunca*
Y *Acacia alata*
Y *Acacia albida*
Y *Acacia anceps*
Y *Acacia aneura*
Y *Acacia arabica (nilotica)*
Y *Acacia arenaria*
Y *Acacia armata*
Y *Acacia aroma*
Y *Acacia aspera*
Y *Acacia ataxacantha*
Y *Acacia aulacocarpa*
Y *Acacia auriculiformis*
Y *Acacia baileyana*
Y *Acacia berlandieri*
Y *Acacia berteriana*
Y *Acacia bidentata*
Y *Acacia biflora*
Y *Acacia blakelyi*
Y *Acacia bonariensis*
Y *Acacia borlaea*
Y *Acacia brachybotrya*
Y *Acacia brachystachya*
Y *Acacia burkei*
Y *Acacia buxifolia*
Y *Acacia byoneana*
Y *Acacia caffra*
Y *Acacia calamifolia*
Y *Acacia calcina*
N *Acacia cambagei*
Y *Acacia cana*
Y *Acacia cardiophylla*
Y *Acacia catechu*
Acacia cavan
Y *Acacia cavenia*
Y *Acacia celastrifolia*
Y *Acacia chariessa*
Y *Acacia cognata*
Y *Acacia colletoides*
Y *Acacia complanata*
Y *Acacia confusa*
Y *Acacia constricta*
Acacia crassicarpa
Y *Acacia cultriformis*
Y *Acacia cunninghamii*
Y *Acacia cupressiformis*
Y *Acacia cyanophylla (saligna)*
Y *Acacia cyclops*
Y *Acacia davyi*
Y *Acacia dealbata*
Acacia deamii
Y *Acacia deanei*
Y *Acacia debora*
Y *Acacia decurrens*
Y *Acacia diptera*
Y *Acacia doratoxylon*
Y *Acacia drummondii*
Y *Acacia ehrenbergiana*
Y *Acacia elata*

Y *Acacia eremophila*
Y *Acacia erioloba*
Y *Acacia erinacea*
Y *Acacia erubescens*
Y *Acacia estrophiolata*
N *Acacia excelsa*
Y *Acacia extensa*
Y *Acacia exuvialis*
Y *Acacia farnesiana*
Y *Acacia filifolia*
Y *Acacia fimbriata*
Acacia fistula
Acacia flava
Y *Acacia fleckii*
Y *Acacia flexuosa*
Y *Acacia floribunda*
Y *Acacia galpinii*
Y *Acacia genistoides*
Y *Acacia georginae*
Y *Acacia giraffae*
Y *Acacia gladiiformis*
Y *Acacia glaucescens*
Y *Acacia glaucoptera*
N *Acacia glomerosa*
Y *Acacia goetzii*
Y *Acacia grandicornuta*
Y *Acacia granitica*
Y *Acacia greggii*
Y *Acacia hakeoides*
Y *Acacia harpophylla*
Y *Acacia harveyi*
Y *Acacia hastulata*
Y *Acacia hebeclada*
Y *Acacia hereroensis*
Acacia heteracantha
Y *Acacia heterophylla*
Y *Acacia holosericea*
Y *Acacia homalophylla*
Y *Acacia horrida*
Y *Acacia horridula*
Y *Acacia huegelii*
Y *Acacia instia*
Y *Acacia jonesii*
Y *Acacia juniperina*
Y *Acacia karoo*
Y *Acacia kauaiensis*
Y *Acacia kempeana*

Y *Acacia kirkii*
Y *Acacia koa*
Y *Acacia koaia*
Y *Acacia kraussiana*
Y *Acacia latifolia*
Y *Acacia leptoneura*
Acacia leucophloea
Y *Acacia linearis*
Y *Acacia lineata*
Y *Acacia lingulata*
N *Acacia loderi*
Y *Acacia longifolia*
Acacia luederitzii
Y *Acacia lunata*
Y *Acacia macrantha*
Y *Acacia macrathyrsa*
Y *Acacia mangium*
Y *Acacia mearnsii*
Y *Acacia melanoxylon*
Y *Acacia mellei*
Y *Acacia mellifera*
Y *Acacia microbotrya*
Y *Acacia mollissima*
Y *Acacia mooreana*
Y *Acacia myrtifolia*
Y *Acacia nebrownii*
Y *Acacia neriifolia*
Y *Acacia nervosa*
Y *Acacia nigrescens*
Y *Acacia nigricans*
Y *Acacia nilotica*
Y *Acacia nubica*
Y *Acacia obliqua*
Y *Acacia obscura*
Y *Acacia orfoto*
Y *Acacia oswaldii*
Y *Acacia parramattensis*
Acacia pence
Y *Acacia pennata*
Y *Acacia pennatula*
Y *Acacia pentadenia*
N *Acacia pentagona*
Y *Acacia permixta*
Y *Acacia podalyriaefolia*
Y *Acacia polyacantha*
Y *Acacia pravissima*
Y *Acacia prominens*

Y *Acacia pubescens*
Y *Acacia pulchella*
Y *Acacia pumila*
Y *Acacia pycnantha*
Y *Acacia raddiana*
Y *Acacia reficiens*
Y *Acacia rehmanniana*
Y *Acacia retiacea*
Y *Acacia rhetinodes*
Y *Acacia richii*
Y *Acacia rigens*
Y *Acacia robusta*
Y *Acacia rostellifera*
Y *Acacia rubida*
Y *Acacia silicina*
Y *Acacia saligna*
N *Acacia schweinfurthii*
Y *Acacia scorpoides*
Y *Acacia senegal*
Y *Acacia seyal*
Acacia siamensis
Y *Acacia sieberana*
Y *Acacia silvicola*
Y *Acacia spadicigera*
Y *Acacia spathulata*
Y *Acacia spinescens*
Acacia spirocarpa
Y *Acacia squamata*
N *Acacia stenophylla*
Y *Acacia stenoptera*
Y *Acacia strigosa*
Y *Acacia stulmanii*
Y *Acacia suavolens*
Y *Acacia subcaerulea*
N *Acacia suffructescens*
Y *Acacia sulcata*
Y *Acacia swazica*
Y *Acacia tamminensis*
Y *Acacia tennispina*
Y *Acacia tetragonocarpa*
Acacia tomentosa
Y *Acacia tortilis*
Y *Acacia triptera*
Y *Acacia tucumanensis*
Y *Acacia unicifera*
Y *Acacia urophylla*
Acacia verek

Y *Acacia verticillata*
Y *Acacia victoriae*
Y *Acacia visco*
Y *Acacia visite*
Y *Acacia volubilis*
Y *Acacia welwitscii*
Y *Acacia xanthophloea*
Y *Acrocarpus fraxinifolius*
N *Adenanthera bicolor*
N *Adenanthera intermedia*
Y *Andenathera pavonina*
N *Afzelia africana*
N *Afzelia quanzensis*
Airyantha borneensis
Airyntha schweinfurthii
Y *Albizia acle*
Y *Albizia adianthifolia*
Y *Albizia amara*
Y *Albizia anthelmintica*
Y *Albizia antunesiana*
Y *Albizia brevifolia*
Y *Albizia carbonaria*
Y *Albizia chinensis*
Y *Albizia distachya*
Y *Albizia ealensis*
Y *Albizia falcataria*
Y *Albizia forbesii*
Y *Albizia glaberrima*
Y *Albizia gummifera*
Y *Albizia harveyi*
Y *Albizia julibrissin*
Y *Albizia katangensis*
Y *Albizia lebbek*
Y *Albizia lebbekoides*
Y *Albiziu lophantha*
Y *Albizia moluccana*
Y *Albizia odoratissima*
Y *Albizia petersiana*
Y *Albizia procera*
Y *Albizia retusa*
Y *Albizia saponaria*
Y *Albizia schimperana*
Y *Albizia stipulata*
Y *Albizia tunganyicensis*
Y *Albizia versicolor*
Y *Albizia zimmermannii*
Aldina insignis
N *Alexa imperatricis*
Y *Alnus acuminata*
Y *Alnus cordata*
Y *Alnus crispa*
Y *Alnus firma*
Y *Alnus formosana*
Y *Alnus fructicosa*
Y *Alnus glutinosa*
Y *Alnus hirsuta*
Y *Alnus incana*
Y *Alnus jorullensis*
Y *Alnus maritima*
Y *Alnus mollis*
Y *Alnus multinervous*
Y *Alnus nepalensis*
Y *Alnus nitida*
Y *Alnus orientalis*
Y *Alnus rubra*
Y *Alnus serrulata*
Y *Alnus sieboldiana*
Y *Alnus sinuata*
Y *Alnus tenuifolia*
Y *Alnus tinctoria*
Y *Alnus undulata*
Y *Alnus viridis*
N *Amblygonocarpus andongensis*
Amburana acreana
Amburana cearensis
Y *Amherstia nobilis*
Amphimas ferrugineus
Anadenanthera colubrina
Y *Anadenanthera peregrina*
Androcalymma glabiforum
Angylocalyx oligophyllus
Angylocalyx zenkeri
Antheroporum pierrei
Anthonotha macrophylla
Apaloxylon madagascariensis
Aphonocalyx cynometroides
Apoplanesia paniculata
Aprevalia floribunda
Apuleia praecox
Arthrocarpum gracile
Arthrosamarea pistaciaefolia
Ateleia pterocarpa
Baikiaea insignis
N *Baikiaea plurijuga*
Baphiopsis parviflora
Barklya syringifolia
Batesia floribunda
Bathiaea rubiflora
Baudouinia sollyiformis
N *Bauhinia acuminata*
N *Bauhinia benthamiana*
N *Bauhinia bidentata*
N *Bauhinia binatà*
N *Bauhinia blakeana*
N *Bauhinia candicans*
N *Bauhinia carronni*
N *Bauhinia corymbosa*
N *Bauhinia cumingiana*
N *Bauhinia diphylla*
N *Bauhinia excisa*
N *Bauhinia galpinii*
N *Bauhinia kirkii*
N *Bauhinia kochiana*
N *Bauhinia kunthiana*
N *Bauhinia macrantha*
N *Bauhinia malabarica*
N *Bauhinia megalandra*
N *Bauhinia monandra*
N *Bauhinia pauletia*
N *Bauhinia petersiana*
N *Bauhinia purpurea*
N *Bauhinia racemosa*
N *Bauhinia reticulata*
N *Bauhinia tomentosa*
Behaimia cubensis
Belairia spinosa
Bergeronia sericea
Berlinia acuminata
Berlinia confusa
N *Berlinia grandiflora*
Y *Bolusanthus speciosus*
N *Bowdichia virgilioides*
N *Brachystegia allenii*
Brachystegia appendiculata
N *Brachystegia boehmii*
Brachystegia glaberrima
N *Brachystegia glaucescens*
Brachystegia kennedyi
N *Brachystegia laurentii*
Brachystegia leonensis
N *Brachystegia manga*
N *Brachystegia microphylla*
Brachystegia nigerica
Y *Brachystegia spiciformis*

N *Brachystegia utilis*
Bragystegia wangermeeana
Brandzeia filicifolia
Breierea insignis
Brongniartia minutifolia
Brongniartia podalyroides
Y *Brownea ariza*
N *Brownea capitella*
N *Brownea coccinea*
N *Brownea crawfordii*
N *Brownea grandiceps*
N *Brownea latifolia*
Browneopsis ucayalina
Y *Brya ebonus*
N *Burkea africana*
Bussea occidentalis
Butea eggelingii
Butea massaiensis
N *Butea monosperma*
Cadia purpurea
N *Caesalpinia cacalaco*
N *Caesalpinia coriaria*
Caesalpinia echinata
Caesalpinia peltophoroides
N *Caesalpinia pulcherrima*
Y *Cajanus cajan*
Y *Calliandra affinis*
Y *Calliandra calothyrsus*
N *Calliandra eriophylla*
Y *Calliandra foliosa*
Y *Calliandra grandiflora*
Y *Calliandra guildingii*
Y *Calliandra haematocephala*
Y *Calliandra haematoma*
N *Calliandra humilis*
Y *Calliandra inaequilatera*
N *Calliandra parvifolia*
Y *Calliandra selloi*
Y *Calliandra surinamensis*
Y *Calliandra tweedii*
Calpocalyx brevibracteatus
Campsiandra angustifolia
Campsiandra comosa
Campsiandra laurifolia
Y *Caragana arborescens*
Y *Caragana aurantiaca*
Y *Caragana frutescens*
Y *Caragana pekinensis*
Cascaronia astragalina
N *Cassia fistula*
N *Cassia grandis*
N *Cassia javanica*
N *Cassia leiandra*
N *Cassia nodosa*
N *Cassia siamea*
Castanospermum australe
Y *Casuarina cristata (C. lepidophloia)*
Y *Casuarina cunninghamiana*
Y *Casuarina equistifolia*
Y *Casuarina fraseriana*
Y *Casuarina glauca*
Casuarina grandis
Y *Casuarina huegeliana*
Y *Casuarina junghuhniana (C. montana)*
Y *Casuarina littoris*
Y *Casuarina muellerana*
Y *Casuarina muricata*
Y *Casuarina nodiflora*
Casuarina obesa
Casuarina ologodon
Y *Casuarina pusilla*
Y *Casuarina quadrivalis*
Y *Casuarina stricta*
Y *Casuarina sumatrana*
Y *Casuarina tenuissima*
Y *Casuarina torulosa*
Y *Cathormion leptophyllum*
Cathormion moniliforme
Y *Ceanothus americanus*
Y *Ceanothus azurens*
Y *Ceanothus cordulatus*
Y *Ceanothus crassifolius*
Y *Ceanothus cuneatus*
Y *Ceanothus delilanus*
Y *Ceanothus divaricatus*
Y *Ceanothus diversifolius*
Y *Ceanothus fendleri*
Y *Ceanothus foliosus*
Y *Ceanothus fresnensis*
Y *Ceanothus glabra*
Y *Ceanothus gloriosa*
Y *Ceanothus greggii*
Y *Ceanothus griseus*
Y *Ceanothus impressus*
Y *Ceanothus incana*

Y *Ceanothus integerrimus*
Y *Ceanothus intermedius*
Y *Ceanothus jepsonii*
Y *Ceanothus leucodermis*
Y *Ceanothus microphyllus*
Y *Ceanothus oliganthus*
Y *Ceanothus ovatus*
Y *Ceanothus parvifolius*
Y *Ceanothus prostratus*
Y *Ceanothus rigidus*
Y *Ceanothus sanguineus*
Y *Ceanothus sorediatans*
Y *Ceanothus thyrsiflorus*
Y *Ceanothus velutinus*
Cedrelinga catenaeformis
Cenostigma macrophyllum
Centrolobium robustum
N *Ceratonia siliqua*
N *Cercidium floridum*
Cercidium praecox
N *Cercidium torreyanum*
N *Cercis siliquastrum*
Y *Cercocarpus betuloides*
Chidlowia sanguinea
Y *Chordospartium stevensonii*
Cladastris kentukia
Cladrastis lutea
N *Cladrastis platycarpa*
Cladrastis sinensis
Y *Clathrotropis brachypetala*
Y *Clathrotropis macrocarpus*
Clathrotropis nitida
N *Colophospermum mopane*
Y *Colvillea racemosa*
Y *Comptonia peregrina (M. asplenifolia)*
Copaifera langsdorfii
Y *Cordeauxia edulis*
N *Cordyla africana*
Y Coriaria angustissima
Y *Coriaria arborea*
Y *Coriaria intermedia*
Y *Coriaria japonica*
Y *Coriaria kingiana*
Y *Coriaria lurida*
Y *Coriaria myrtifolia*
Y *Coriaria plumosa*
Y Coriaria pottsiana
Y *Coriaria pteridoides*
Y *Coriaria sarmentosa*
Y *Coriaria thymifolia*
Y *Craibia baptisarum*
Y *Craibia brevicaudata*
Craibia grandiflora
Crudia gabonensis
N *Crudia parivoa*
Cyclobium brasiliense
Cyclolobium vecchii
Cylicodiscus gabunensis
Cymbosepalum baroni
Cynometra alexandri
Cynometra ananta
Cynometra bauhiniaefolia
N *Cynometra cauliflora*
N *Cynometra hankei*
Cynometra leonensis
N *Cynometra ramiflora*
Cynometra retusa
Dalbergia baroni
Dalbergia cearensis
Dalbergia cochinchinensis
Dalbergia cubilquitensis
Dalbergia greveana
Y *Dalbergia latifolia*
Y *Dalbergia melanoxylon*
Dalbergia nigra
Dalbergia retusa
Y *Dlbergia sissoo*
Dalbergia spruciana
Dalbergia stevensonii
Y *Dalbergiella nyasae*
Y *Dalea spinosa*
Daniellia ogea
Daniellia olivera
Daniellia thurifera
Dansera procera
Delaportea armata
Delonix baccal
N *Delonix elata*
Y *Delonix regia*
Denistophytum madagascariense
Y *Derris indica*
Detarium senegalense
Y *Dewevrea bilabiata*
N *Dialium engleranum*

N *Dialium pachyphyllum*
Y *Dialium zenkeri*
Y *Dichrostachys cinerea*
Y *Dichrostachys glomerata (D.cinera)*
Y *Dichrostachys spicata*
Dicorynia guianensis
Dicraeopetalum stipulare
Y *Dicymbe altsoni*
Y *Dicymbe corymbosa*
Didelotia africana
Y *Dimorphandra davisii*
Dinizia excelsa
Diphysa floribunda
Diphysa robinioides
N *Diplotropis purpurea*
Dipteryx odorata
Dipteryx trifoliata
Diptychandra epunctata
Y *Discaria toumatou*
Distemonanthus benthamianus
Y *Dryas drummondii*
Y *Dryas integrifolia*
Y *Dryas octopetalia*
Duparquetia orchidacea
Dussia discolor
Dussia martinicensis
Y *Elaeagnus angustifolia*
Y *Elaeagnus argentea*
Y *Elaeagnus commutata*
Y *Elaeagnus edulis*
Y *Elaeagnus longipes*
Y *Elaeagnus macrophylla*
Y *Elaeagnus multiflora*
Y *Elaeagnus pungens*
Y *Elaeagnus rhamnoides*
Y *Elaeagnus umbellata*
Eligmocarpus cyometroides
Elizabetha durissima
Elizabetha princeps
Endertia spectabilis
Englerodendrom usambarense
Y *Entada abyssinica*
Y *Entada phaseoloides*
Y *Entada sudanica (E. africanum)*
Y *Enterolobium cyclocarpum*
Y *Enterolobium schomburgkii*
Y *Enterolobium timbouva*
Y *Eperua falcata*
Eperua jenmani
Eperua purpurea
Y *Erythrina abyssinica*
Y *Erythrina americana*
Y *Erythrina berteroana*
Y *Erythrina caffra*
Y *Erythrina crista-galli*
Y *Erythrina fusca*
Y *Erythrina glauca*
Y *Erythrina indica*
Y *Erythrina lithosperma*
Y *Erythrina monosperma*
Erythrina orientalis
Y *Erythrina poeppigiana*
Y *Erythrina suberosa*
Y *Erythrophleum africanum*
Erythrophleum ivorense
Y *Erythrophleum suaveolens*
Etaballia dubia
Europetalum batesii
Eurypatalum tessmanii
Exostyles venusta
N *Eysenhardtia amorphoides*
Eysenhardtia peninsularis
Y *Eysenhardtia texana*
Ferreirea spectabilis
Fillaeopsis discophora
Fissicalyx fendleri
Fordia cauliflora
Gagnebina tamariscina
Y *Genista sp.*
Y *Geoffroea decorticans*
Geoffroea spinosa
Gilbertiodendron demonstrans
Gilletiodendron klanei
N *Gleditsia amorphoides*
N *Gleditsia caspica*
N *Gleditsia japonica*
N *Gleditsia sinensis*
N *Gleditsia triacanthos*
Gliricidia ehrenbergii
Gliricidia lambii
Y *Gliricidia sepium*
Goldmania foetida
Y *Gossweilerodendron balsamiferum*
Y *Gourliea decorticans*

N *Guibourtia coleosperma*
N *Guibourtia conjugata*
Guibourtia demeusei
Guibourtia schliebenii
N *Gymnocladus dioicus*
Haematoxylon brasiletto
N *Haematoxylon campechianum*
Haplormorsia monophylla
Hardwickia binata
Y *Hardwickia pinnata*
Harpalyce cubensis
Hebestigma cubense
Hesperolaburnum platycarpum
Hesperothamnus littoralis
Heterostemon mimosoides
Y *Hippophae rhamnoides*
N *Holocalyx balansae*
Humboldtia laurifolia
Hylodendrom gabunense
Hymenaea confertiflora
Y *Hymenaea courbaril*
Hymenolobium excelsum
Hymenolobium nitidum
Hymenostegia floribunda
Indopiptadenia oudhensis
Inga altissima
Y *Inga edulis*
Inga feullei
Y *Inga jiniquil*
Y *Inga laurina*
Y *Inga oerstediana*
Inga paterna
Y *Inga vera*
N *Inocarpus edulis*
N *Intsia acuminata*
N *Intsia bakeri*
Y *Intsia bijuga*
Intsia palembanica
Intsia plurijuga
Intsia retusa
Isoberlina schefflera
Isoberlinia argotensis
Isoberlinia dalzielii
Isoberlinia doka
Isoberlinia tomentosa
Isomacrolobium leptorrachis
Jacqueshuberia quinquangulata
N *Julbernardia globiflora*
Julbernardia hochreutineri
Julbernardia magnistipulata
Julbernardia paniculata
Julbernardia seretti
Julbernardia unijugata
Kalappia celebica
Kingiodendron alternifolium
Kingiodendron pinnatum
Koompassia excelsa
N *Koompassia malaccensis*
Y *Laburnum alpinum*
Y *Laburnum anagyroides*
Y *Laburnum pratense*
Lebruniodendron lepthanthum
Lecointea amazonica
Lennea robinioides
Leonardoxa africana
Y *Leucaena collinsii*
Y *Leucaena diversifolia*
Y *Leucaena esculenta*
Y *Leucaena lanceolota*
Y *Leucaena leucocephala*
Y *Leucaena macrophylla*
Y *Leucaena pulverulenta*
Y *Leucaena retusa*
Y *Leucaena shannoni*
Y *Leucaena trichodes*
Leucostegane latistipulata
Librevillea klainei
Loesenera kalantha
Y *Lonchocarpus capassa*
Y *Lonchocarpus latifolius*
Lonchocarpus punctatus
Lonchocarpus utilis
Y *Lonchocarpus violaceus*
Y *Lysidice rhodostegia*
Lysiloma auritum
Lysiloma bahamensis
Lysiloma divaricata
Lysiloma latisiliqua
Y *Lysiloma thornberi*
Y *Maackia amurensis*
N *Maackia chinensis*
Maackia floribunda
Y *Machaerium robinifolium*
Machaerium schomburgkii
Macroberlinia bracteosa

Y *Macrozamia communis*
Y *Macrozamia riedlei*
Y *Maniltoa grandiflora*
N *Maniltoa scheffera*
Marmaroxylon racemosum
Martiodendron excelsum
Melanoxylon brauna
Michelsonia microphylla
Microberlinia brazzavillensis
Milbraediodendron excelsum
Y *Millettia dubia*
Millettia grandis
Millettia laurentii
Millettia rubiginosa
Y *Millettia stuhlmannii*
Y *Millettia thonningii*
Y *Millettia usaramensis*
Mimosa bracaatinga
Y *Mimosa scabrella*
Y *Mimosa tenuiflora*
Mimozyganthus carinatus
Moldenhauera floribunda
Monopetalanthus pteridophyllus
Monopteryx angustifolia
Monoschisma leptostachyum
Y *Mora excelsa*
Mora gonggrijpii
Muellera frutescens
Y *Mundulea sericea*
Y *Myrica adenophora*
Y *Myrica asplenifolia*
Y *Myrica carolinensis*
Y *Myrica cerifera*
Y *Myrica gale*
Y *Myrica javanica*
Y *Myrica pensylvanica*
Y *Myrica pilulifera*
Y *Myrica pubescens*
Y *Myrica rubra*
Y *Myrica sapida*
Y *Myrica serrata*
Myrocarpus fastigiatus
Y *Myrocarpus frondosus*
Myrospermum frutescens
Y *Myroxylon balsamum*
Myroxylon pereirae
Myroxylon peruiferum
Neochevalierodendron stephanii
Neodunnia atrocyanea
Neoharmsia madagascariensis
N *Newtonia buchananii*
N *Newtonia hildebrandtii*
Notodon gracilis
Notospartium glabrescens
Oddoniodendron micranthum
Oleiocarpon panamense
Y *Olneya tesota*
Y *Ormosia coccinea*
Ormosia hosei
Y *Ormosia monosperma*
Y *Ostryoderris gabonica*
Y *Ostryoderris stuhlmannii*
Y *Ougeinia oojeinensis*
Oxystigma mannii
Oxystigma msoo
Pachyelasma tessmannii
Pahudia galedupa
N *Pahudia rhomboidea*
Paloue guianensis
Paloveopsis emarginata
Panurea longifolia
Paramachaerium schomburgkii
N *Paramacrolobium coeruleum*
Y *Parapiptadenia rigida*
Y *Parasponia andersonii*
Y *Parasponia parviflora*
Y *Parasponia rugosa*
Y *Parkia africana*
Y *Parkia biglandulosa*
Parkia biglobosa
Parkia clappertoniana
Parkia filicoidea
Y *Parkia javanica*
Y *Parkia roxburghii*
Y *Parkia especiosa*
Parkia timoriana
Y *Parkinsonia aculeata*
Parkinsonia africana
Pellegriniodendron diphyllum
Peltogyne catingue
Peltogyne densiflora
Peltogine excelsa
Peltophorum adnatum
Peltophorum dasyrrhachis
N *Peltophorum pterocarpum*

25

	Peltophorum vogelianum
	Pentaclethra eetveldeana
Y	*Pentaclethra macroloba*
	Pentaclethra macrophylla
Y	*Pericopsis angolensis*
Y	*Pericopsis elata*
	Pericopsis mooniana
	Petaladenium urceoliferum
Y	*Phyllocarpus riedelii*
	Phyllocarpus septentrionalis
	Phylloxylon xiphoclada
	Phylloxylon xyiophylloides
	Pictetia aculeata
N	*Piliostigma malabaricum*
	Piliostigma reticulatum
N	*Piliostigma thonningii*
	Piptadenia excelsa
	Piptadenia macrocarpa
	Piptadenia paraguaynensis
	Piptadeniastrum africanum
Y	*Piscidia piscipula*
Y	*Pithecellobium adinocephalum*
	Pithecellobium arboreum
Y	*Pithecellobium caraboboense*
Y	*Pithecellobium cauliflorum*
Y	*Pithecellobium collinum*
Y	*Pithecellobium dulce*
	Pithecellobium flexicaule
N	*Pithecellobium jiringa*
Y	*Pithecellobium lanceolatum*
	Pithecellobium lobatum
	Plagiosiphon discifer
	Plathymenia reticulata
	Platycelyphium cynanthum
	Platycyamus regnellii
	Platycyamus ulei
	Platymiscium dimorphandrum
Y	*Platymiscium pinnatum*
Y	*Platymiscium trinitatis*
	Platymiscium ulei
	Platypodium elegans
	Platysepalum vanhouttei
	Platysepalum violaceum
	Podopetalum ormondii
	Poecilanthe effusa
	Poeppigia procera
	Pogocybe entadoides
	Polystemonanthus dinklagei
Y	*Pongamia pinnata*
	Prioria copaifera
Y	*Prosopis africana*
Y	*Prosopis alba*
Y	*Prosopis articulata*
Y	*Prosopis chilensis*
Y	*Prosopis cineraria*
Y	*Prosopis dulcis*
Y	*Prosopis glandulosa*
Y	*Prosopis juliflora*
Y	*Prosopis kuntzeie*
Y	*Prosopis nigra*
Y	*Prosopis pallida*
Y	*Prosopis ruscifolia*
Y	*Prosopis tamarugo*
Y	*Prosopis velutina*
	Pseudosamanea guachapele
Y	*Psorodendron spinosum*
	Pterocarpus angolensis
	Pterocarpus blancoi
Y	*Pterocarpus echinatus*
Y	*Pterocarpus indicus*
Y	*Pterocarpus marsupium*
Y	*Pterocarpus officinalis*
Y	*Pterocarpus podocarpus*
Y	*Pterocarpus rotundifolius*
	Pterocarpus santaloides
	Pterocarpus sericeus
Y	*Pterocarpus soyauxii*
	Pterocarpus stevensonii
Y	*Pterocarpus vidalianus*
	Pterodon emarginatus
N	*Pterogyne nitens*
	Pynaertiodendron congolanum
	Ramorinoa girolae
	Recordoxylon amazonicum
Y	*Robinia hispida*
	Robinia neomexicana
Y	*Robinia pseudoacacia*
Y	*Robinia viscosa*
	Sabinea florida
	Sakoanala madagascariensis
	Samanea pedicellaris
	Samanea polycephala
Y	*Samanea saman*
	Samanea saminiqua
N	*Saraca asoca*
Y	*Saraca declinata*

N *Saraca indica*
Saraca palembanica
N *Saraca thaipingensis*
Saraca trianda
Schefflerodendron usambarense
Y *Schizolobium parahyba*
Schizoscyphus roseus
Schotia afra
N *Schotia brachypetala*
N *Schotia capitata*
N *Schotia latifolia*
Y *Sclerolobium aureum*
Sclerolobium chrysolobium
Y *Sclerolobium micropetalum*
Scorodophloeus fischeri
N *Scorodophloeus zenkeri*
Serianthes dilmyi
Serianthes myriadenia
Sesbania aegyptica
Y *Sesbania arborea*
Y *Sesbania cinerascens*
Sesbania formosa
Y *Sesbania grandiflora*
Sesbania punctata
Y *Sesbania roxburghii*
Y *Sesbania sesban*
Y *Shepherdia argentea*
Y *Shepherdia canadensis*
Sindora coriacea
Sindora inermis
Sindora intermedia
Sindora javanica
N *Sindora supa*
Sindora wallachii
Sindoropsis le-testui
Y *Sophora chrysophylla*
Y *Sophora flavescens*
Y *Sophora japonica*
Sophora linearifolia
Sophora macrocarpa
Y *Sophora tetraptera*
Y *Sophora tomentosa*
Soprosis palmeri
Spirotropis longifolia
Stachyothyrsus staudtii
Stahlia maritima
Steinbachiella leptoclada
Stemonocoleus micranthus
Storkiella vitiensis
Strombocarpa strombuliferra
Y *Stryphnodendron adstringens*
Y *Stryphnodendron barbatimam*
Stuhlmannia moavi
Swartzia fistuloides
Swartzia guianensis
Y *Swartzia madagascariensis*
Y *Swartzia trinitensis*
Sweetia elegans
Sweetia fruticosa
Sweetia nitens
Sweetia panamensis
Y *Sweetia praeclara*
Sympetalandra borneensis
Tachigalia paniculata
Talbotiella gentii
N *Tamarindus indica*
Terua vallicola
Tessmannia africana
Tessmannia demiflora
Tetraberlinia bifoliolata
Tetrapleura tetraptera
Tetrapteracarpon geayi
Y *Tipuana tipu*
Y *Trachylobium verrucosum*
Uittienia modesta
Uleanthus erythrinoides
Umtiza listeriana
Uribea tamarindoides
Vatairea guianensis
Vataireopsis araroba
Y *Virgilia capensis*
Virgilia divaricata
Vouacapoua americana

Y *Wallaceodendron celebicum*
Willardia mexicana
Xanthocercis madagascariensis
Y *Xanthocercis zambesiaca*
Y *Xeroderris stuhlmannii*
Xylia evansii
Xylia ghesquierei
Xylia xylocarpa
Yucaratonia brenningii
Zenia insignis
Zenkerella citrina
Zollernia falcata